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National Research Council
Research Associateship Programs

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EVALUATION OF ASSOCIATE BY RESEARCH ADVISER

Jonathan F. Smith, Ph.D.
Name of Research Adviser

U.S. Army Medical Research Institute of Infectious Diseases
Address of Laboratory
Fort Detrick
Frederick, MD 21702-5011

Peter Pushko, Ph.D.
Name of Associate & Idnumber

[✓ Regular?] or Senior?
[✓ Renewal?] or Final?

Original starting date

(1) How long and in what capacity have you known the Associate?

I first corresponded with Dr. Pushko two years ago, and have known him personally for approximately one year during the time he has been an associate in my laboratory.

(2) Give a brief statement of the Associate's research program, and comment on the progress and principal accomplishments, including publications, during tenure of the Associateship. If this is to support an application for renewal, state why the renewal is necessary or desirable. (SEE CONTINUATION SHEET)

The primary objectives of Dr. Pushko's research is to design, construct, and evaluate a new vaccine delivery system based on alphavirus replicons. Previously, we have developed a new live-attenuated vaccine candidate for Venezuelan equine encephalitis (VEE) by incorporating attenuating mutations into "infectious" cDNA clones by site-directed mutagenesis. The positive strand nature of the alphavirus genome allows us to recover infectious virus from cDNA clones by

(3) The purpose of the Associateship programs administered by the National Research Council is to provide to postdoctoral scientists and engineers of unusual ability and promise an opportunity for research on problems largely of their personal choice which may contribute to the general research effort of the host laboratory. Comment below to what degree the above purpose was, or is being, fulfilled.

Dr. Pushko is clearly an exceptional scientist, and his progress on the vaccine vector systems has been remarkable. However, because of the high biocontainment required for work with VEE virus, there are few other laboratories in which he would have been able to pursue this work. One of the major objectives of USAMRIID is the development of new vaccines to viral, bacterial, and toxin agents. If Dr. Pushko's work is successful, it could significantly enhance vaccine efforts throughout the Institute.

(EVALUAT1 91)

EXHIBIT H

EVALUATION OF ASSOCIATE BY RESEARCH ADVISOR (Continued)

Research Advisor: Jonathan F. Smith
NRC Associate: Peter Pushko

2. Statement of Associate's research program (continued):

transcription and transfection of cells in culture. It is also possible to produce alphavirus transcripts which contain all of the nonstructural genes, but in which the structural genes are replaced with any gene of interest. Such transcripts, or replicons, are self-replicating and express the heterologous gene(s) at high levels. If the viral structural genes are provided on a second "helper" RNA, which is cotransfected with the first, the recombinant replicon is packaged *in trans* by the structural proteins expressed from the helper.

In theory, such packaged replicons could be used as vaccine vectors, which would produce a single cycle of infection and expression *in vivo*. A fundamental problem existed, however, insofar as recombination between replicon and helper RNA's regenerated fully infectious virus. Dr. Pushko's research was designed to make such a vaccine vector system from the VEE vaccine cDNA clone, in which recombination would yield, at worse, a vaccine designed for human use. Secondly, his research was designed to validate the assumption that vaccination with single cycle replicons would induce an immune response to the heterologous gene. Thirdly, he intended to produce a replicon-helper system which would preclude the formation of complete virus by recombination. Dr. Pushko's research has been impressive and successful. He has succeeded in producing replicon-helper systems from the attenuated VEE clone, and has demonstrated that this system will express heterologous genes (Lassa N and influenza HA) to high levels. Furthermore, he has shown that inoculation of packaged replicons containing the Lassa N gene into mice induces a prompt antibody response to this protein.

After only six months working on this project, Dr. Pushko has already submitted an abstract of his progress to an international meeting. However, Dr. Pushko has observed the formation of complete VEE virus in his system, presumably as a result of recombination. Although safe, vaccinated recipients would develop an immunity to the vector system, and thereby preclude repeated use in the same individual. A second year is therefore requested to allow the construction of modified replicon-helper systems to overcome this problem.